Skeletal muscle phenotype is associated with exercise tolerance in patients with peripheral arterial disease

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Objective: **To better understand the association between skeletal muscle and exercise intolerance in peripheral arterial disease (PAD), we assessed treadmill-walking performance and gastrocnemius muscle phenotype in healthy control subjects and in patients with PAD. We hypothesized that gastrocnemius muscle characteristics would be altered in PAD compared with control subjects and that exercise tolerance in patients PAD would be related to muscle phenotype.**

Methods: **Sixteen patients with PAD and intermittent claudication and 13 healthy controls of the same age participated. Each subject completed a graded treadmill-walking test and underwent a resting muscle biopsy. Muscle biopsy samples were obtained from the medial gastrocnemius muscle of the most ischemic limb in PAD and a limb chosen at random in controls. Samples were analyzed for fiber type and cross-sectional area, capillary-to-fiber ratio, the number of capillaries in contact with each fiber type, and the optical density of glycogen within each fiber by using histochemical procedures. Total muscle glycogen content was determined biochemically.**

Results: **Exercise capacity measured on the incremental walking test in the PAD group was only 30% to 40% of that observed in controls. The PAD group had a lower proportion of type I muscle fibers ($P < .05$), fewer capillaries per muscle fiber ($P < .05$), and tended to have smaller fiber areas ($P = .08$). The relative area of type I fibers, the capillary-to-fiber ratio, capillary contacts with type I and IIa fibers, and the optical density of glycogen in type I fibers were all positively correlated with exercise tolerance in the PAD group($P < .05$) but not controls.**

Conclusions: **These data suggest that muscle phenotype is altered in PAD and that such alterations are associated with the exercise intolerance in these patients. In light of these findings, therapies such as resistance training or electrical stimulation that target skeletal muscle in PAD may prove beneficial, and further investigation of such therapies is warranted. (J Vasc Surg 2005;41:802-7.)**

It is well known that peripheral arterial disease (PAD), resulting in intermittent claudication, is associated with a reduced exercise tolerance. Intolerance to common modes of exercise like walking can reduce levels of daily physical activity, lead to poor health, and impair quality of life.[1] The mechanisms underlying the functional impairment in these patients are not fully understood, however.[2]

PAD is primarily a vascular disease, with the major limitation being a restriction of arterial blood flow to the lower limbs. Some evidence indicates that physiologic changes within the skeletal muscle may also contribute to the functional impairment,[3] although the nature of these alterations is not clear. Some investigators have found gastrocnemius muscle fiber areas to be smaller in PAD,[4,5] whereas others have found no difference between PAD patients and healthy control subjects.[1,6] Similarly, data in relation to capillary supply and fiber type distribution are conflicting, with no clear picture of whether these characteristics are altered in PAD.[1,4-10] Differences in muscle carbohydrate metabolism amongst claudicants have also been observed.[11] The impact of PAD on muscle phenotype is clearly an important area of research that requires clarification. Furthermore, there is a need to establish what role these physiologic alterations might have on exercise capacity by exploring the relationship between muscle phenotype and exercise capacity needs to be established.

We aimed to further describe the gastrocnemius muscle characteristics of PAD patients and a group of well-matched healthy control subjects. In particular, we examined the muscle fiber type distribution, cross-sectional area, capillary supply, and glycogen content and hypothesized that these muscle characteristics would be significantly altered in PAD patients compared with control subjects. A novel aspect of this study was the investigation of the relationship between muscle phenotype and exercise capacity, and we hypothesized that the various muscle fiber characteristics would be significantly correlated with walking capacity in PAD.

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METHODS

Recruitment and screening. Subjects gave informed consent to the experimental procedures, which were approved by the local ethics committee. Sixteen individuals with PAD, defined as a resting ankle-to-brachial systolic blood pressure index (ABI) <.9 and a history of stable intermittent claudication >1 year, were included. Thirteen healthy control subjects with no history of chronic disease also participated. The ratio of men to women in each group was the same ($z = 0.71$, $P = .48$) (Table I). Subjects with uncontrolled hypertension (>180 mm Hg systolic), uncontrolled angina, or diabetes were excluded. All subjects from both groups were sedentary in that they did not engage in any regular physical activity and had completed no more than one bout of continuous exercise (≥10 minutes) in any given 7-day period in the previous 12 months.[12] For each leg in all subjects, the ABI was calculated using the highest systolic ankle pressure and the highest brachial artery pressure. The "worse" leg of the PAD patients was defined as that with the lowest post-exercise ABI. Subject characteristics are presented in Table I.

Graded treadmill test. After being familiarized with testing procedures, subjects performed a graded treadmill test on a motorized treadmill (Payne, Australia) at 3.2 km/h. The treadmill grade was set at 0% for the first 5 minutes and then increased by 3.5% every 3 minutes until subjects reached their maximum. Maximal exercise time was recorded and peak (vertical) power on the treadmill was calculated using the equation: Peak power (W) = (grade/100) · body mass · 9.81 · 0.89, where *peak power* is the highest power generated in translating the center of mass in the vertical direction and is measured in watts *(W)*, *grade* is treadmill elevation measured as percent incline, *body mass* is measured in kg, $9.81\ m \cdot s^{-2} \cdot s^{-1}$ is the acceleration due to gravity, and *0.89* is the walking speed measured in m/s.

Heart rate (HR) was recorded with a Vantage portable monitor (Polar Electro-Oy, Finland) and averaged over 15-second intervals. Pulmonary gas exchange measurements of minute ventilation (V_E), oxygen uptake (VO_2), and respiratory exchange ratio were made every 15 seconds using the Q-Plex automated gas analysis system (Quinton, Seattle). ABI was measured before and after the graded treadmill test as per the method described under *Recruitment and screening.*

Muscle characteristics. The calf muscle mass of each subject was estimated using anthropometrical measurements.[13] Seventy-two hours after the graded treadmill test, a resting muscle biopsy was performed using a 6-mm bore percutaneous biopsy needle. After 30 minutes of rest, the muscle biopsy was taken from a consistent location of the medial gastrocnemius of the worse leg in PAD patients and a randomly selected leg in the controls. The sample was immediately divided, and one piece was frozen in liquid nitrogen and stored at −80°C for the biochemical determination of muscle glycogen.[14] The other piece was prepared for histochemical analysis by orienting the tissue in a longitudinal fashion before immersing it in Tissue-Tek embedding medium (Sakura Finetek, USA) and freezing it in precooled isopentane.

Table I. Subject characteristics

	Controls	*PAD*
No. of subjects	13	16
Men	8	13
Height (cm)	170 (2)	168 (1)
Weight (kg)	75 (4)	73 (3)
Age (years)	62 (2)	63 (2)
Brachial systolic pressure (mm Hg)	123 (3)	140 (5)*
Resting ABI $_{\text{worse leg}}$	—	0.58 (0.03)*
Resting ABI $_{\text{better leg}}$	—	0.75 (0.03)*
Resting ABI $_{\text{dominant}}$	1.28 (0.02)	—
Resting ABI $_{\text{nondominant}}$	1.29 (0.02)	—

PAD, Peripheral arterial disease; *ABI,* ankle-to-brachial index.
*Significantly different from controls ($P < .05$).

For histochemical analyses, five serial cross sections of the tissue were cut with a cryostat at −20°C and mounted on cover slips, each in triplicate (15 sections in total). Three of the sections (10 μm thickness) were stained for myosin adenosine triphosphatase to identify type I (slow oxidative), IIa (fast oxidative), and IIb (fast glycolytic) fibers.[15]

The cross-sectional area of each fiber was measured with MCID image analysis software (Image Research Inc, St. Catherines, Ontario, Canada). The two remaining sections (16 μm) underwent periodic acid-Schiff (PAS) and PAS-amylase staining to identify glycogen and capillaries, respectively.[16] Digital microphotographs of the glycogen PAS stains were converted to 8-bit images (255 gray scale), and integrated optical density measures (expressed as gray level; 0 = black and 255 = white) were made within each fiber. The optical density of glycogen in each fiber, relative to the background optical density, was then calculated using the equation: $100 \times \log^{10}$ (background density/fiber density).[17]

The total capillary-to-fiber ratio was calculated by dividing the number of capillaries by the number of fibers counted. The number of capillaries in contact with each individual fiber (i.e., capillary contacts) was also counted. One control and two PAD subjects chose not to have a muscle biopsy. In addition, low tissue yields from some of the biopsies meant that a full complement of data was not available for all the histochemical analyses (PAD, n = 9 to 14; control, n = 10 to 12).

Statistics. Muscle characteristics were compared using a two-way (group-by-fiber type) analysis of variance and differences were identified using Tukey's test. All other variables were compared between the groups using unpaired *t* tests. The Pearson product moment correlation coefficient was used to describe relationships between variables. Statistical significance was set at $P \leq .05$. Data are expressed as mean ± SEM.

Table II. Peak performance and physiological data from the graded treadmill-walking test in controls and PAD subjects

	Controls	*PAD*
Maximal walking time (s)	1505 (62)	495 (57)*
Peak power (W)	179 (12)	73 (5)*
Peak VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	37.4 (2.0)	16.7 (1.1)*
Peak V_E (L/min)	109 (9)	49 (4)*
Peak RER	1.19 (0.03)	1.05 (0.02)*
Peak HR	169 (4)	120 (6)*
Postexercise ABI†	1.08 (0.02)	0.23 (0.02)*

PAD, Peripheral arterial disease; RER, respiratory exchange ratio; *HR,* heart rate; *ABI,* ankle-brachial index.
*Significantly different from controls ($P < .05$).
†Postexercise ABI is from randomly selected limb in control subjects and the worse limb in PAD patients (ie, the same limb that underwent muscle biopsy).

RESULTS

Maximal walking time and peak power attained on the graded treadmill test were both lower in PAD patients than in controls (Table II). Peak VO_2, post exercise ABI, and other physiologic responses on the treadmill test were also significantly lower in the PAD group than in controls (Table II).

There was no difference in estimated calf muscle mass between the better (1538 ± 62 g) and worse (1516 ± 68 g) PAD legs; both of these measures were less than that observed in controls (1760 ± 71 g; $P < .05$). Gastrocnemius muscle fiber data (Table III) are based on an average fiber count of 176 ± 17 and 195 ± 18 in controls and PAD, respectively. PAD muscle had a higher proportion of type IIa, and a lower proportion of type I fibers than controls. PAD muscle fibers tended to have smaller, by about 9%, cross-sectional areas than controls ($P = .08$). This finding was not affected by removing women from the analysis.

The relative area of muscle occupied by type I fibers was significantly lower in PAD (49% ± 4% vs 64% ± 5%; $P < .05$) and, for type IIa fibers, relatively higher in PAD (29% ± 3% vs 16% ± 3%; $P < .05$). The relative area of type IIb fibers did not differ between the groups (PAD, 22% ± 4%; control, 19% ± 4%).

Gastrocnemius capillary-to-fiber ratio tended to be lower in PAD subjects than in control ($P = .08$), and PAD subjects had fewer capillary contacts per fiber (Table III). The magnitude of this difference, which was 15%, was not altered when women were removed from the comparison. When capillary-to-fiber ratio was expressed relative to average fiber area, the groups did not differ significantly (PAD, 353 ± 19; CON, 404 ± 29; $P = .15$). Muscle glycogen content did not differ between PAD subjects and controls. There was a significant fiber type effect where the relative optical density of glycogen was lower in type I fibers than type II fibers in PAD and controls (Table III).

Peak treadmill power in the PAD group was significantly correlated with the capillary contacts of type I (Fig 1) and IIa ($r = .70$) fibers but not type IIb. In addition, muscle glycogen content measured biochemically was significantly correlated with treadmill peak power in PAD ($r = .55$), as was the density of glycogen in type I fibers (Fig 2). The cross-sectional area of type I fibers was significantly correlated with peak treadmill power in PAD subjects ($r = .72$) and controls ($r = .66$). The strength of association between maximal exercise time or peak VO_2 and several other muscle characteristics in the PAD group are listed in Table IV. Neither resting nor postexercise ABI was correlated with walking capacity or any of the muscle phenotype variables in either group.

DISCUSSION

PAD is associated with a reduced exercise tolerance, and this was reaffirmed in the present study where the walking capacity of PAD subjects was only 30% to 40% of that in control subjects. We found several alterations in the gastrocnemius muscle of PAD patients that were significantly correlated with maximal walking capacity in the PAD group but not in the controls. That the resting and postexercise ABI was not correlated with walking capacity highlights the potential importance of skeletal muscle to exercise intolerance in claudicants and the need to develop and test novel therapies that target skeletal muscle in PAD.

The gastrocnemius muscle is particularly important to study in PAD, because it is the most common site of ischemic pain that limits exercise tolerance.[18] Control values for the distribution of type I fibers (60%), capillary-to-fiber ratio (2.1), and capillary contacts (4.7) fell within the ranges previously reported for healthy men and women in this age group.[5-8,19,20]

Calf muscle mass was reduced by 12% in the PAD group compared with control subjects, confirming previous observations in PAD patients.[1,21] Such reductions in calf muscle mass are thought to be due to muscle fiber atrophy in PAD,[4,5,21] which was also observed in the present study (ie, 9% lower, $P = .08$).

PAD muscle had fewer type I fibers (48%) than did control. Consistent with the lower number of oxidative type I fibers, estimates of capillary supply were also 20% to 25% lower in PAD subjects. Our data on these muscle characteristics are in strong agreement with those reported by Clyne et al,[7] who studied the gastrocnemius medialis muscle in 60 claudicants and 10 to 12 healthy controls.

Capillary rarefaction in skeletal muscle is a feature of hypertension,[22] and hypertensive patients also have a lower proportion of type I fibers (48%) compared with normotensive individuals (57%).[23] Therefore, the altered muscle phenotype in PAD patients may be directly attributable to their hypertensive state (Table I), although no clear relationship between blood pressure and muscle characteristics was evident in the present study.

Alternatively, common pathologic processes may explain the skeletal muscle changes in individuals with hypertension and those with PAD. Physical activity is also known to alter skeletal muscle phenotype. Although we attempted to only enroll sedentary subjects, it is possible that PAD subjects were habitually less active than control subjects

Table III. Skeletal muscle characteristics in controls and PAD subjects

		Controls	*PAD*
Fiber type (%)	I	62 (4)	49 (5)*
	IIa	16 (3)	27 (3)*
	IIb	22 (5)	24 (4)
Fiber CSA (μm^2)	I	5407 (292)	4842 (400)
	IIa	5407 (292)	4842 (400)
	IIb	4980 (528)	4423 (458)
	Total	5308 (350)	4649 (413)
Capillary-to-fiber ratio		2.1 (0.2)	1.6 (0.2)†
Capillary contacts	I	4.9 (0.3)	4.2 (0.3)
	IIa	4.4 (0.3)	4.0 (0.4)
	IIb	3.8 (0.2	3.4 (0.2)
	Total	4.7 (0.3)	4.0 (0.3)‡
Capillary contacts *10^6/fiber CSA	I	976 (70)	942 (61)
	IIa	943 (115)	872 (57)
	IIb	1008 (152)	835 (69)
	Total	955 (68)	911 (52)
Relative optical density of glycogen (%)	I	25.5 (1.2)	28.0 (1.2)§
	IIa	31.3 (1.5)	32.5 (1.6)
	IIb	31.0 (0.9)	31.2 (1.4)
	Total	27.4 (1.6)	29.7 (1.1)
Glycogen content (mmol/kg d.w.)		351 (46)	361 (35)

PAD, Peripheral arterial disease; *CSA*, cross-sectional area.
*Significantly different from controls ($P < .05$).
†Significantly different from controls ($P = .08$).
‡Significant main effect between PAD and control for capillary contacts.
§Significant main effect between type I and type IIa and IIb fibers for glycogen density ($P < .05$).

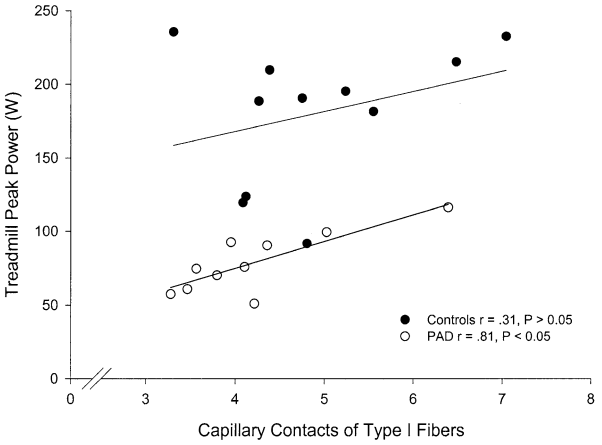

Fig 1. The relationship between the capillary contacts of type I fibers and peak power during treadmill walking in control subjects *(CON)* and peripheral arterial disease *(PAD)* subjects.

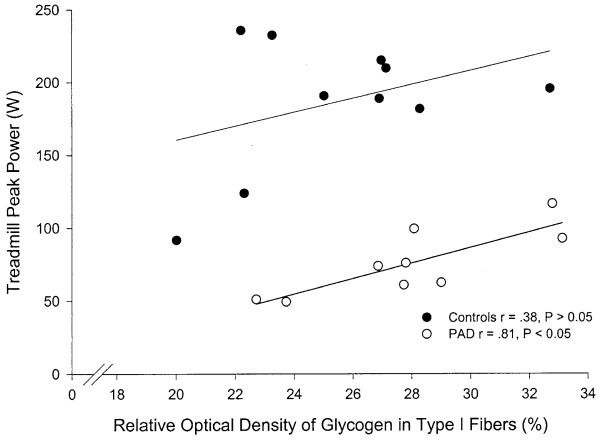

Fig 2. The relationship between the relative optical density of glycogen (PAS stain intensity) in type I fibers and peak power during treadmill walking in control subjects *(CON)* and peripheral arterial disease *(PAD)* subjects.

and this contributed to the observed differences in muscle characteristics. However, there is evidence that physical activity levels of older PAD patients are not different from that of healthy control subjects.[5] Furthermore, in unilaterally diseased patients where activity levels are self-controlled, it is confirmed that muscle mass is reduced in the diseased limbs compared with asymptomatic limbs.[1]

Supervised exercise therapy has also been shown to have no effect on muscle mass and on type I fiber distribution in claudicants.[24,25] However, there is some evidence in PAD that resistance training increases muscle capillary supply and fiber cross-sectional area.[24] Further research is required to understand the effect of habitual physical activity and exercise on muscle phenotype in claudicants.

There are conflicting data in relation to the effect of PAD on muscle phenotype. Although our data agree with some studies, our findings do differ with others. Muscle capillarization in PAD has been found to be lower, similar, or higher than control values,[5-9] and a wide range of type I fiber percentages have been observed across the different

Table IV. Correlation coefficients for selected relationships between maximal exercise time or VO_2 peak and muscle fiber characteristics in the peripheral arterial disease worst limb

	%Area type I	*%Area type IIa*	*%Area type IIb*	*C-F ratio*	*CC type I*	*ROD-G type I*
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	.73*	−.09	−.64*	.65*	.61*	.50
Maximal exercise time (s)	.57*	−.23	−.40	.72*	.66*	.61†

PAD, Peripheral arterial disease; *C-F,* capillary-to-fiber; *CC,* capillary contacts; *ROD-G,* Relative optical density of glycogen.
*Significant at $P \leq .05$.
†Significant at $P \leq .10$.

PAD groups studied (48% to 65%).[1,5,7,8] Varying degrees of hypertension or different physical activity levels, as described above, might contribute to the variant muscle phenotype findings in the literature.

The wide range of findings in PAD might also reflect the variation in its severity or duration, or both. As PAD symptoms progress in severity from claudication to rest pain, the composition of skeletal muscle shifts towards a greater proportion of type I fibers.[4,10,26] This might be related to either the preferential loss of type II fibers in more severe disease[4,21] or fiber remodeling,[4] or both, that occurs in response to fiber denervation.[1,27]

Methodologic problems such as low sample sizes ($N \leq 5$)[6,10] may also account for some of the divergent findings. In some studies, muscle samples have been obtained from open biopsies during surgery under general anesthesia, and findings may not be comparable to others, including this study, where a percutaneous biopsy has been performed under local anesthesia. An accurate histochemical analysis of skeletal muscle requires ≥100 fibers,[28] whereas several PAD studies have used as few as 50 fibers.[6] Finally, variations in the age and gender composition of the groups might have contributed to the divergent findings among PAD studies. In the present study, we tried to avoid as many of these problems as possible, by taking large biopsy samples, counting a large number of fibers in triplicate, and comparing groups of the same age and similar gender composition.

Considerable debate has emerged about the pathophysiology of exercise intolerance in PAD, with a particular focus on the roles of hemodynamic limitations versus changes intrinsic to skeletal muscle.[29] There is uncertainty about their influences on exercise intolerance,[29,30] and the main aim of the present study was to further explore the relationship between skeletal muscle phenotype and exercise capacity. The potential importance of the differences in skeletal muscle fiber type, fiber area, and capillarization observed in the present study to exercise intolerance in PAD are supported by the strong correlations between these same variables and walking capacity (Table IV, Fig 1). In addition, glycogen content in whole muscle and type I muscle fibers (Fig 2) were positively correlated with walking performance. These histologic and biochemical data add to other reports of significant correlations between the metabolic characteristics of skeletal muscle and exercise tolerance in claudicants.[25,31-34] That such correlations were not observed in the controls is a consistent finding among these studies and suggests these skeletal muscle characteristics may be important to understanding exercise intolerance in PAD.

Although correlations should not imply causality, several of our findings suggest the following scenario of how differences in skeletal muscle phenotype might contribute to a reduced walking capacity. Calf pain usually limits walking in claudicants,[18] and we assume that calf muscle fatigue occurs more rapidly[35] and contributes to exercise intolerance in claudicants compared with healthy controls. Given that PAD muscle has more fatigable type II fibers (than type I fibers), the recruitment of a greater number of type II muscle fibers during walking is more likely and so a greater rate of fatigue is more probable. The likelihood of recruiting more type II muscle fibers is further increased by the smaller calf muscle mass and, particularly, smaller muscle fiber area; because for a given force output, more muscle fibers should be recruited in accordance with the size principle (type I to type IIb). The lower capillarization of all fiber types in PAD will render them more vulnerable to hypoxia and, thus, fatigue.[36,37] The positive correlations between walking performance and type I glycogen content in PAD suggests that lower muscle glycogen levels in those fibers first recruited during walking (type I) would predispose them to earlier failure and hasten the recruitment of additional, more fatigable fibers.[38]

Although further research is required, these data suggest that the differences in muscle characteristics we have observed might contribute to a greater recruitment of more fatigable muscle fibers and thus lead to the reduced exercise capacity in individuals with PAD. Alternatively, exercise intolerance caused by claudication might lead to a reduction in habitual physical activity. This, in-turn, might contribute to the altered muscle phenotype and could explain the association between muscle characteristics and exercise capacity in PAD. As already discussed, however, the effect of variations in physical activity on muscle phenotype in PAD needs to be confirmed.

Therapies targeting skeletal muscle, such as resistance training or electrical stimulation, promote angiogenesis and a fast-to-slow transition in muscle fibers in the presence of improved fatigue resistance.[24,39] Such therapies have received little attention in PAD, and in light of our findings, further investigation of their use is warranted.

In conclusion, PAD muscle was characterized by a smaller muscle fiber size, lower percentage of type I fibers, and a lower capillary supply than healthy controls. These variables, as well as the glycogen density of type I fibers, were also positively correlated with walking capacity in PAD only. Although there is a need to confirm if a cause-and-effect relationship exists among these variables, these data suggest that skeletal muscle phenotype is associated with exercise intolerance in PAD and highlight the need to develop and test therapies that target skeletal muscle.

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